



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/70, 39/39, C07H 21/00		A2	(11) International Publication Number: WO 00/61151
			(43) International Publication Date: 19 October 2000 (19.10.00)

(21) International Application Number: PCT/US00/09839	(22) International Filing Date: 12 April 2000 (12.04.00)	(30) Priority Data: 60/128,898 12 April 1999 (12.04.99) US	(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(71) Applicant (<i>for all designated States except US</i>): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).	(72) Inventors; and	(73) Inventors/Applicants (<i>for US only</i>): KLINMAN, Dennis [US/US]; 2 Candlelight Court, Potomac, MD 20854 (US). ISHII, Ken [JP/US]; 257 Congressional Lane #120, Rockville, MD 20852 (US). VERTHELYI, Daniela [AR/US]; 11615 Regency Drive, Potomac, MD 20854 (US).	Published <i>Without international search report and to be republished upon receipt of that report.</i>
(74) Agents: GAGALA, Bruce, M. et al.; Leydig, Voit & Mayer, Ltd., Suite 4900, Two Prudential Plaza, 180 North Stetson, Chicago, IL 60601-6780 (US).			

(54) Title: OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

(57) Abstract

The present invention provides a substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by either the formula: 5' N₁N₂N₃T-CpG-WN₄N₅N₆ 3' wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides, or the formula: 5' RY-CpG-RY 3' wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T, as well as an oligodeoxynucleotide delivery complex and a pharmacological composition comprising the present inventive oligodeoxynucleotide, and a method of inducing an immune response by administering the present inventive oligodeoxynucleotide to a host.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates generally to induction of an immune response using specific oligodeoxynucleotides (ODNs).

BACKGROUND OF THE INVENTION

DNA is a complex macromolecule whose immunological activities are 10 influenced by its base composition and base modification, as well as helical orientation. Certain unusual DNA structures (e.g., Z-DNA) can induce significant antibody responses when administered to normal mice. In addition, bacterial DNA, as well as certain synthetic ODNs containing unmethylated CpG sequences can induce 15 proliferation and immunoglobulin (Ig) production by murine B cells. Unmethylated CpG dinucleotides are more frequent in the genomes of bacteria and viruses than 20 vertebrates. Recent studies suggest that immune recognition of these motifs may contribute to the host's innate immune response. D.M. Klinman et al., *CpG Motifs Present in Bacterial DNA Rapidly Induce Lymphocytes to Secrete Interleukin 6, Interleukin 12, and Interferon γ* , 93 Proc. Natl. Acad. Sci. USA 2879 (1996); A.-K. Yi et al., *Rapid Immune Activation by CpG Motifs in Bacterial DNA*, 157 J. Immun. 5394 (1996); Hua Liang et al., *Activation of Human B Cells by Phosphorothioate Oligodeoxynucleotides*, 98 J. Clin. Invest. 1119 (1996); A.M. Krieg et al., *CpG Motifs in Bacterial DNA Trigger Direct B-Cell Activation*, 374 Nature 546 (1995).

In mice, CpG DNA induces proliferation in almost all (>95%) of B cells and 25 increases Ig secretion. This B-cell activation by CpG DNA is T-cell independent and antigen non-specific. In addition to its direct effects on B cells, CpG DNA also directly activates monocytes, macrophages, and dendritic cells to secrete a variety of cytokines. These cytokines stimulate natural killer (NK) cells to secrete γ -interferon (IFN- γ) and have increased lytic activity. Examples of which can be found in 30 International Patent Applications WO 95/26204, WO 96/02555, WO 98/11211, WO 98/18810, WO 98/37919, WO 98/40100, WO 98/52581, PCT/US98/047703, and PCT/US99/07335; U.S. Patent No. 5,663,153; and U.S. Patent Applications Serial

Nos. 08/276,358, 08/386,063, 08/461,036, 08/462/799, 08/960,774, 08/738,652, 09/030,701, 09/082,649, 09/191,170, 09/09/136,138, 09/154,614, and 09/286,098.

Although bacterial DNA and certain ODNs can induce a murine immune response, little is known about the immunostimulatory capacity of these materials for the human immune system. Z.K. Ballas et al., *Induction of NK Activity in Murine and Human Cells by CpG Motifs in Oligodeoxynucleotides and Bacterial DNA*, 157 J. Immun. 1840 (1996). Differences in the responsiveness of human and murine B cells to certain stimuli render it impossible to extrapolate results obtained from mouse to man.

10 In view of the above, there exists a need for ODNs that induce an immune response in humans. In addition, there is a need for methods utilizing ODNs in the treatment of human diseases. The present invention provides such ODNs and methods of use. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention

15 provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a substantially pure or isolated ODN of at least about 10 nucleotides comprising a sequence represented by either the formula:

20



wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides, or the formula:

25



wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T. The present invention also provides an ODN delivery complex and pharmacological composition comprising the present inventive ODN, as well as a method of inducing an immune response by administering the present inventive ODN to a host.

DETAILED DESCRIPTION OF THE INVENTION

Oligodeoxynucleotide

The present invention provides novel ODNs. These ODNs have at least about 10 nucleotides and comprise a sequence represented by either the formula:

5



wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides, or the formula:

10



wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T. For example, the ODN can be selected from the group consisting of SEQ ID NO: 1 15 through SEQ ID NO: 99.

Preferably, the ODN of the present invention is substantially pure or isolated. "Substantially pure" refers to an ODN that is substantially free of other materials, particularly other nucleic acids, proteins, lipids, carbohydrates, and other materials with which it may be naturally associated, while "isolated" refers to an ODN that is 20 removed from its natural environment or state. Preferably, the ODN of the present invention consists of about 100 nucleotides or less (e.g., about 10-75 nucleotides). More preferably, the ODN consists of about 50 nucleotides or less (e.g., about 10-40 nucleotides). Even more preferably, the ODN consists of about 30 nucleotides or less (e.g., about 10-20 nucleotides). Most preferably the ODN consists of about 12 to 25 about 16 nucleotides.

Any suitable modification can be used in the present invention to render the ODN resistant to degradation *in vivo* (e.g., via an exo or endonuclease). Preferably, the modification includes a phosphorothioate modification. The phosphorothioate modifications can occur at either termini, e.g., the last two or three 5' and/or 3' 30 nucleotides can be linked with phosphorothioate bonds. The ODN also can be modified to contain a secondary structure (e.g., stem loop structure) such that it is resistant to degradation. Another modification that renders the ODN less susceptible

to degradation is the inclusion of nontraditional bases such as inosine and quanine, as well as acetyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine. Other modified nucleotides include nonionic DNA analogs, such as alkyl or aryl phosphonates (i.e., the charged phosphonate oxygen is replaced 5 with an alkyl or aryl group, as set forth in U.S. Patent No. 4,469,863), phosphodiesters and alkylphosphotriesters (i.e., the charged oxygen moiety is alkylated, as set forth in U.S. Patent No. 5,023,243 and European Patent No. 0 092 574). ODNs containing a diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini, have also been shown to be more resistant to degradation.

10 Preferably, the ODNs inducing a humoral immune response, e.g., 5' N₁N₂N₃T-CpG-WN₄N₅N₆ 3', contain a phosphate backbone modification, and more preferably, the phosphate backbone modification is a phosphorothioate backbone modification (i.e., one of the non-bridging oxygens is replaced with sulfur, as set forth in International Patent Application WO 95/26204). For the ODNs 15 inducing a cell-mediated immune response and containing a phosphodiester backbone, e.g., 5' RY-CpG-RY 3', the ODN preferably has been modified to prevent degradation.

Oligodeoxynucleotide Delivery Complex

20 The present inventive oligodeoxynucleotide delivery complex comprises the present inventive ODN and a targeting means. Any suitable targeting means can be used within the context of the present invention.

An ODN can be associated with (e.g., ionically or covalently bound to, or 25 encapsulated within) a targeting means (e.g., a molecule that results in higher affinity binding to a target cell, such as a B cell). A variety of coupling or cross-linking agents can be used to form the delivery complex, such as protein A, carbodiamide, and N-succinimidyl-3-(2-pyridylthio) propionate (SPDP). Examples of ODN delivery complexes include ODNs associated with a sterol (e.g., cholesterol), a lipid (e.g., a cationic lipid, virosome or liposome), and a target cell specific binding agent 30 (e.g., a ligand recognized by target cell specific receptor). Preferred complexes must be sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization

by the target cell; however, these complexes can be cleavable under appropriate circumstances such that the ODN can be released in a functional form.

Pharmacological Composition

5 The present inventive pharmacological composition comprises the present inventive ODN and a pharmacologically acceptable carrier. Pharmacologically acceptable carriers (e.g., physiologically or pharmaceutically acceptable carriers) are well known in the art.

10 The present inventive pharmacological composition facilitates the use of the present inventive ODN, both *in vivo* and *ex vivo*. Such a composition can be suitable for delivery of the active ingredient to any suitable host, such as a patient for medical application, and can be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

15 Pharmacological compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more pharmacologically (e.g., physiologically or pharmaceutically) acceptable carriers comprising excipients, as well as optional auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper
20 formulation is dependent upon the route of administration chosen. Thus, for injection, the active ingredient can be formulated in aqueous solutions, preferably in physiologically compatible buffers. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the active
25 ingredient can be combined with carriers suitable for inclusion into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For administration by inhalation, the active ingredient is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant. The active ingredient can be formulated for parenteral
30 administration by injection, e.g., by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or

aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Other pharmacological excipients are known in the art.

Method of Inducing an Immune Response

5 The present inventive method of inducing an immune response comprises administering the present inventive ODN to a host in order to induce an immune response in the host.

Administration of the present inventive ODN can be by any suitable method. For example, the ODN can be administered *in vivo* or *ex vivo*. Preferably, the ODN is 10 administered *in vivo* to a mammal, particularly a human. Optionally, the ODN can be contained within or conjugated with a protein, hydrocarbon or lipid. Once this molecule is administered, the ODN sequence must be exposed on the surface to induce an immune response. The ODN can also be co-administered with a protein, hydrocarbon, or lipid. Co-administration can be such that the ODN is administered 15 before, at substantially the same time as, or after the protein, hydrocarbon, or lipid. Preferably, the ODN is administered at substantially the same time as the protein, hydrocarbon, or lipid.

After administration of the novel ODNs, while not intending to be bound by any particular theory, it is thought that the ODNs initially act on antigen presenting 20 cells (e.g., macrophages and dendritic cells). These cells then release cytokines, which activate natural killer (NK) cells. Either a cell-mediated or humoral immune response then occurs in the host.

The cell-mediated or local immune response is produced by T cells, which are able to detect the presence of invading pathogens through a recognition system 25 referred to as the T-cell antigen receptor. Upon detection of an antigen, T cells direct the release of multiple T-cell cytokines, including IL-2, IL-3, IFN- γ , TNF- β , GM-CSF and high levels of TNF- α , and chemokines MIP-1 α , MIP-1 β , and RANTES. IL-2 is a T-cell growth factor that promotes the production of additional T cells sensitive to the particular antigen. This production constitutes a clone of the T cells. The sensitized 30 T cells attach to cells containing the antigen. T cells carry out a variety of regulatory and defense functions and play a central role in immunologic responses. When stimulated to produce a cell-mediated immune response, some T cells respond by

acting as killer cells, killing the host's own cells when these cells are infected or cancerous and therefore recognized as foreign. Some T cells respond by stimulating B cells, while other T cells respond by suppressing immune response. Preferably, if a cell-mediated immune response is induced, non-B cells are activated, more 5 preferably, cytokines are produced, and most preferably, IFN- γ is produced.

The humoral or systemic immune response depends on the ability of the B cells to recognize specific antigens. The mechanism by which B cells recognize antigens is through specific receptors on the surface of the B cells. When an antigen attaches to the receptor site of a B cell, the B cell is stimulated to divide. The 10 daughter cells become plasma cells that manufacture antibodies complementary to the attached antigen. Each plasma cell produces thousands of antibody molecules per minute, which are released into the bloodstream. Many B cells appear to be regulated by the helper T cells and suppressor T cells and produce various cytokines, e.g., IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF and low levels of TNF- α . Helper T 15 cells stimulate B cells to produce antibodies against antigens, while suppressor T cells inhibit antibody production. Some B cells, however, are T-cell independent and require no stimulation by the T cells. Preferably, if a humoral immune response is induced, B cells are activated, more preferably, IL-6 is produced, and most preferably, antibodies are produced.

20 In addition, induction of one type of immune response may allow for immune regulation because up regulation of one type of immune response may down regulate the other type of immune response. This immune regulation allows for customizing or tailoring of the type of immune response when administering an ODN.

The present inventive method can be used to treat, prevent, or ameliorate any 25 suitable allergic reaction in combination with any suitable anti-allergenic agent. An allergy, in the context of the present invention, refers to an acquired hypersensitivity to a substance (i.e., an allergen). Allergic conditions include eczema, allergic rhinitis or coryza, hay fever, bronchial asthma, urticaria (hives), food allergies, and other atopic conditions. The list of allergens is extensive and includes pollens, insect 30 venoms, animal dander, dust, fungal spores, and drugs (e.g., penicillin). Examples of natural, animal, and plant allergens can be found in International Patent Application WO 98/18810. Preferably, the present inventive method is used to treat allergic

asthma. Suitable anti-allergenic agents include those substances given in treatment of the various allergic conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to treat any suitable cancer in combination with any suitable anti-cancer agent. Suitable cancers include cancers of the brain, lung (e.g., small cell and non-small cell), ovary, breast, prostate, and colon, as well as carcinomas and sarcomas. Preferably, the present inventive method is used to treat a solid tumor cancer. Suitable anti-cancer agents include those substances given in treatment of the various conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to improve the efficacy of any suitable vaccine. Suitable vaccines include those directed against Hepatitis A, B, and C, examples of which can be found in the Physicians' Desk Reference (1998), and DNA vaccines directed against HIV and malaria. *See generally* D. Klinman et al., *CpG Motifs as Immune Adjuvants*, 17 Vaccine 19 (1999); M.J. McCluskie and H.L. Davis, *CpG DNA is a Potent Enhancer of Systemic & Mucosal Immune Response Against Hepatitis B Surface Antigen with Intra-Nasal Administration to Mice*, 161 J. Immun. 4463 (1998).

The present inventive method can be used to treat, prevent, or ameliorate any suitable disease associated with the immune system. Preferred diseases associated with the immune system are autoimmune disorders and immune system deficiencies, e.g., lupus erythematosus, and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. Immune system deficiencies include those diseases or disorders in which the immune system is not functioning at normal capacity, or in which it would be useful to boost the immune system response.

The present inventive method can be used with any suitable antisense therapy. Suitable antisense agents are those that bind either with DNA or RNA and block their function by inhibiting expression of the sequence to which the antisense agents are bound. *See generally* H. Lonnberg et al., *Towards Genomic Drug Therapy with Antisense Oligonucleotides*, 28 Ann. Med. 511 (1996); A. Alama et al., *Antisense Oligonucleotides as Therapeutic Agents*, 36 Pharmacol. Res. 171 (1997); K.J. Scanlon et al., *Oligonucleotide-Mediated Modulation of Mammalian Gene Expression*, 9

FASEB J. 1288 (1995); R. Oberbauer, *Not Non-Sense but Antisense – Applications of Antisense Oligonucleotides in Different Fields of Medicine*, 109 Wien Klin Wochenschr 40 (1997).

The present inventive method can be used to treat, prevent, or ameliorate any 5 suitable infection in combination with any suitable anti-infectious agent. Examples include francisella, schistosomiasis, tuberculosis, AIDS, malaria, and leishmania. Examples of suitable infectious viruses, bacteria, fungi, and other organisms (e.g., protists) can be found in International Patent Application WO 98/18810. Suitable anti-infectious agents include those substances given in treatment of the various 10 conditions described elsewhere, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent. Suitable bio-warfare agents include those naturally occurring biological agents that have been specifically 15 modified in the laboratory. Often, modification of these agents has altered them such that there is no known treatment. Examples include Ebola, Anthrax, and Listeria. In the course of ameliorating the symptoms after exposure, use of the present inventive ODNs may not cure the patient, but rather can extend the patient's life sufficiently such that some other treatment can then be applied.

20 The present invention is further described in the following examples. These examples are intended only to illustrate the invention and are not intended to limit the scope of the invention in any way.

EXAMPLES

25 *Example 1*

The following example demonstrates induction of an immune response by various ODNs. Induction was measured by production of the cytokines IL-6 and TNF- γ , and cell proliferation.

Human peripheral blood mononuclear cells (PBMC) were isolated, as 30 described elsewhere (Z.K. Ballas et al., 85 J. Allergy Clin. Immunol. 453 (1990); Z.K. Ballas and W. Rasmussen, 45 J. Immunol. 1039 (1990); Z.K. Ballas and W. Rasmussen, 150 J. Immunol. 17 (1993)). ODNs were synthesized on a DNA

synthesizer (Applied Biosystems Inc., Foster City, CA), as described elsewhere (Beacage and Caruthers, *Deoxynucleoside Phosphoramidites – A New Class of Key Intermediates for Deoxypolynucleotide Synthesis*, 22 Tetrahedron Letters 1859 (1981)). In some ODNs, the normal DNA backbone phosphodiesterase linkages were 5 replaced with phosphorothioate linkages, as described elsewhere (Agrawal et al., 94 Proc. Natl. Acad. Sci. USA 2620 (1997); Agrawal 14 TIB TECH 376 (1996)). To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs. IL-6 and TNF- γ levels 10 were determined by ELISA using anti-IL-6 and anti-TNF- γ antibodies, as described elsewhere (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). Cell proliferation was determined by [3 H] thymidine incorporation, as described elsewhere (Liang et al., 98 J. Clin. Invest. at 1121).

15 IL-6 levels and cell proliferation are set forth in Table 1: Induction of a Humoral Immune Response *In Vitro*. These data demonstrate that a sequence containing 5' N₁N₂N₃T-CpG-WN₄N₅N₆ 3', wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides, is desirable to induce a humoral immune response. In addition, maximum induction was 20 observed for ODNs that contained a phosphorothioate backbone. IFN- γ levels and cell proliferation are set forth in Table 2: Induction of a Cell-Mediated Immune Response *In Vitro*. These data demonstrate that a sequence containing 5' RY-CpG-RY 3', wherein the central CpG motif is unmethylated, R is A or G and Y is C or T, is desirable to induce a cell-mediated immune response. Maximum induction occurred 25 with ODNs containing phosphodiesterase linkages.

Table 1. Induction of a Humoral Immune Response *In Vitro*.

	IL-6 Levels (ELISA)	Cell Proliferation (3 H Thymidine Incorporation)
SEQ ID NO: 1	65	52
SEQ ID NO: 2	85	44
SEQ ID NO: 3	54	50

	IL-6 Levels (ELISA)	Cell Proliferation (³ H Thymidine Incorporation)
SEQ ID NO: 4	48	61
SEQ ID NO: 5	42	100
SEQ ID NO: 6	55	23
SEQ ID NO: 7	35	69
SEQ ID NO: 8	28	38
SEQ ID NO: 9	41	20
SEQ ID NO: 10	42	16
SEQ ID NO: 11	33	77
SEQ ID NO: 12	25	13
SEQ ID NO: 13	28	13
SEQ ID NO: 14	35	67
SEQ ID NO: 15	28	54
SEQ ID NO: 16	39	50
SEQ ID NO: 17	50	32
SEQ ID NO: 18	26	1
SEQ ID NO: 19	12	2
SEQ ID NO: 20	55	92
SEQ ID NO: 21	53	26
SEQ ID NO: 22	8	2
SEQ ID NO: 23	12	1
SEQ ID NO: 24	14	0
SEQ ID NO: 25	30	42
SEQ ID NO: 26	43	60
SEQ ID NO: 27	17	15
SEQ ID NO: 28	14	0
SEQ ID NO: 29	10	1
SEQ ID NO: 30	28	23
SEQ ID NO: 31	16	17

Table 2. Induction of a Cell-Mediated Immune Response *In Vitro*.

	IFN- γ Levels (ELISA)	Cell Proliferation (³ H Thymidine Incorporation)
SEQ ID NO: 32	78	1
SEQ ID NO: 33	100	2
SEQ ID NO: 34	73	2
SEQ ID NO: 35	88	4

	IFN- γ Levels (ELISA)	Cell Proliferation (3 H Thymidine Incorporation)
SEQ ID NO: 36	81	5
SEQ ID NO: 37	45	4
SEQ ID NO: 38	78	0
SEQ ID NO: 39	33	5
SEQ ID NO: 40	68	2
SEQ ID NO: 41	54	2
SEQ ID NO: 42	54	1
SEQ ID NO: 43	74	4
SEQ ID NO: 44	53	4
SEQ ID NO: 45	32	9
SEQ ID NO: 46	24	1
SEQ ID NO: 47	23	8
SEQ ID NO: 48	22	25
SEQ ID NO: 49	34	26
SEQ ID NO: 50	36	8
SEQ ID NO: 51	24	17
SEQ ID NO: 52	21	9
SEQ ID NO: 53	19	2
SEQ ID NO: 54	12	8
SEQ ID NO: 55	15	5
SEQ ID NO: 56	22	6
SEQ ID NO: 57	18	3
SEQ ID NO: 58	18	6
SEQ ID NO: 59	12	21
SEQ ID NO: 60	13	4
SEQ ID NO: 61	--	2
SEQ ID NO: 62	12	23
SEQ ID NO: 63	16	1
SEQ ID NO: 64	16	4
SEQ ID NO: 65	19	2
SEQ ID NO: 66	16	4
SEQ ID NO: 67	14	2
SEQ ID NO: 68	13	1
SEQ ID NO: 69	12	2
SEQ ID NO: 70	19	2
SEQ ID NO: 71	13	1
SEQ ID NO: 72	14	46
SEQ ID NO: 73	--	4
SEQ ID NO: 74	16	1
SEQ ID NO: 75	24	1

	IFN- γ Levels (ELISA)	Cell Proliferation	
		^3H Thymidine Incorporation)	
SEQ ID NO: 76	13	1	
SEQ ID NO: 77	12	1	
SEQ ID NO: 78	13	1	
SEQ ID NO: 79	13	1	
SEQ ID NO: 80	12	1	
SEQ ID NO: 81	14	20	
SEQ ID NO: 82	14	43	
SEQ ID NO: 83	14	1	
SEQ ID NO: 84	12	1	
SEQ ID NO: 85	15	2	
SEQ ID NO: 86	13	1	
SEQ ID NO: 87	12	0	
SEQ ID NO: 88	--	3	
SEQ ID NO: 89	15	1	
SEQ ID NO: 90	18	2	
SEQ ID NO: 91	13	2	
SEQ ID NO: 92	12	1	
SEQ ID NO: 93	14	2	
SEQ ID NO: 94	14	1	
SEQ ID NO: 95	44	3	
SEQ ID NO: 96	24	1	
SEQ ID NO: 97	21	6	
SEQ ID NO: 98	36	38	
SEQ ID NO: 99	21	26	

The foregoing data demonstrates the induction of an immune response in human cells, as exemplified by PBMC, and as measured by the production of the cytokines IFN- γ and IL-6, and cell proliferation, occurs upon the administration of various ODNs.

Example 2

The following example demonstrates induction of an immune response *ex vivo* by various ODNs. Induction was measured by production of the cytokine IL-6.

10 A human B cell line (RPMI 8226) was maintained according to the manufacturers recommendations. ODNs were synthesized as described in Example 1. In some ODNs, the normal DNA phosphodiesterate linkages were replaced with

phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the ends. The cells were incubated with various ODNs for 14 hrs. IL-6 production was determined by ELISA using anti-IL-6 antibodies, as 5 described in Example 1.

IL-6 levels are set forth in Table 3: Induction of a Humoral Immune Response *Ex Vivo*. These data confirm that a sequence containing 5' N₁N₂N₃T-CpG-WN₄N₅N₆ 3', which are linked by phosphorothioate bonds and wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides, is 10 desirable to induce a humoral immune response.

Table 3. Induction of a Humoral Immune Response *Ex Vivo*.

IL-6 Levels (ELISA)	
SEQ ID NO: 1	100
SEQ ID NO: 2	89
SEQ ID NO: 3	85
SEQ ID NO: 4	82
SEQ ID NO: 5	82
SEQ ID NO: 6	78
SEQ ID NO: 7	78
SEQ ID NO: 8	78
SEQ ID NO: 9	73
SEQ ID NO: 10	65
SEQ ID NO: 11	62
SEQ ID NO: 12	58
SEQ ID NO: 13	57
SEQ ID NO: 14	56
SEQ ID NO: 15	50
SEQ ID NO: 16	48
SEQ ID NO: 17	47
SEQ ID NO: 18	45
SEQ ID NO: 19	40
SEQ ID NO: 20	39
SEQ ID NO: 21	33
SEQ ID NO: 22	25
SEQ ID NO: 23	23

15	
IL-6 Levels (ELISA)	
SEQ ID NO: 24	21
SEQ ID NO: 25	18
SEQ ID NO: 26	17
SEQ ID NO: 27	17
SEQ ID NO: 28	16
SEQ ID NO: 29	16
SEQ ID NO: 30	13
SEQ ID NO: 31	13

The foregoing data demonstrates the induction of an immune response in human cells, as exemplified by the human B cell line RPMI 8226, and as measured by production of the cytokine IL-6, occurs upon administration of various ODNs.

5

The following table lists additional ODNs which fall within the scope of the present invention.

Table 4:

10

SEQ ID NO: 100
SEQ ID NO: 101
SEQ ID NO: 102
SEQ ID NO: 103
SEQ ID NO: 104
SEQ ID NO: 105
SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110
SEQ ID NO: 111
SEQ ID NO: 112
SEQ ID NO: 113
SEQ ID NO: 114
SEQ ID NO: 115
SEQ ID NO: 116
SEQ ID NO: 117

SEQ ID NO: 118
SEQ ID NO: 119
SEQ ID NO: 120
SEQ ID NO: 121
SEQ ID NO: 122
SEQ ID NO: 123
SEQ ID NO: 124
SEQ ID NO: 125
SEQ ID NO: 126
SEQ ID NO: 127
SEQ ID NO: 128
SEQ ID NO: 129
SEQ ID NO: 130
SEQ ID NO: 131
SEQ ID NO: 132
SEQ ID NO: 133
SEQ ID NO: 134
SEQ ID NO: 135
SEQ ID NO: 136
SEQ ID NO: 137
SEQ ID NO: 138
SEQ ID NO: 139
SEQ ID NO: 140
SEQ ID NO: 141
SEQ ID NO: 142
SEQ ID NO: 143

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by the following formula:

5



wherein the central CpG motif is unmethylated, W is A or T, and N₁, N₂, N₃, N₄, N₅, and N₆ are any nucleotides.

10

2. A substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by the following formula:



15

wherein the central CpG motif is unmethylated, R is A or G and Y is C or T.

3. The oligodeoxynucleotide of claim 2, wherein the sequences on the 5' side of the CpG sequences form a palindrome with the sequences on the 3' side of the 20 CpG sequence.

4. The oligodeoxynucleotide of any of claims 1-3, wherein the oligodeoxynucleotide is selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 143.

25

5. The oligodeoxynucleotide of any of claims 1-4, wherein the oligodeoxynucleotide is modified to prevent degradation.

6. The oligodeoxynucleotide of any of claims 1-5, wherein the 30 oligodeoxynucleotide has a phosphate backbone modification.

7. The oligodeoxynucleotide of claim 6, wherein the phosphate backbone modification is a phosphorothioate backbone modification.
8. The oligodeoxynucleotide of any of claims 1-7, wherein the 5 oligodeoxynucleotide comprises about 100 nucleotides or less.
9. The oligodeoxynucleotide claim 8, wherein the oligodeoxynucleotide comprises about 50 nucleotides or less.
10. The oligodeoxynucleotide of claim 9, wherein the 10 oligodeoxynucleotide comprises about 30 nucleotides or less.
11. The oligodeoxynucleotide of claim 10, wherein the 15 oligodeoxynucleotide comprises about 12-16 nucleotides.
12. An oligodeoxynucleotide delivery complex comprising the oligodeoxynucleotide of any of claims 1-11 and a targeting means.
13. The oligodeoxynucleotide delivery complex of claim 12, wherein the 20 targeting means is selected from the group consisting of cholesterol, virosome, liposome, lipid, and a target cell specific binding agent.
14. A pharmacological composition comprising the oligodeoxynucleotide of any of claims 1-11 and a pharmacologically acceptable carrier.
- 25
15. A method of inducing an immune response in a host comprising administering to a host an oligodeoxynucleotide of any of claims 1-11.
16. The method of claim 15, wherein the immune response induced is a 30 cell-mediated immune response.

17. The method of claim 15 or 16, wherein the oligodeoxynucleotide activates non-B cells in the host.
18. The method of any of claims 15-17, wherein the oligodeoxynucleotide induces cytokine production in the host.
19. The method of claim 18, wherein the cytokine is IFN- γ .
20. The method of claim 15, wherein the immune response induced is a humoral immune response.
21. The method of claim 15 or 20, wherein the oligodeoxynucleotide activates B cells in the host.
- 15 22. The method of any of claims 15, 20, or 21, wherein the oligodeoxynucleotide induces IL-6 production in the host.
- 20 23. The method of any of claims 15, 20-22, wherein the oligodeoxynucleotide induces antibody production in the host.
24. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate an allergic reaction, and the oligodeoxynucleotide is administered either alone or in combination with an anti-allergenic agent.
- 25 25. The method of claim 24, wherein the allergic reaction is asthmatic.
26. The method of any of claims 15-23, wherein the induction of an immune response is used to treat cancer, and the oligodeoxynucleotide is administered either alone or in combination with an anti-cancer agent.
- 30 27. The method of claim 26, wherein the cancer is a solid tumor cancer.

28. The method of any of claims 15-23, wherein the induction of an immune response is used to improve the efficacy of a vaccine, and the oligodeoxynucleotide is administered either alone or in combination with a vaccine.

5

29. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent or ameliorate a disease associated with the immune system.

10

30. The method of claim 29, wherein the disease associated with the immune system is an autoimmune disorder.

31. The method of claim 29, wherein the disease associated with the immune system is an immune system deficiency.

15

32. The method of any of claims 15-23, wherein the induction of an immune response is used in antisense therapy, and the oligodeoxynucleotide is administered either alone or in combination with an antisense agent.

20

33. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate an infection, and the oligodeoxynucleotide is administered either alone or in combination with an anti-infectious agent.

25

34. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent.

30

35. The method of any of claims 15-34, wherein the method further comprises:

(a) administering the oligodeoxynucleotide to lymphocytes *ex vivo*, thereby producing activated lymphocytes, and

(b) administering the activated lymphocytes obtained in step (a) to the host.

36. The method of any of claims 15-34, wherein the host is a human.

1
SEQUENCE LISTING

<110> Klinman, Dennis
Verthelyi, Daniela
Ishii, Ken

<120> OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

<130> 175900

<140> US

<141> 1999-04-12

<160> 143

<170> PatentIn Ver. 2.0

<210> 1

<211> 12

<212> DNA

<213> synthetic

<400> 1

tcgagcggttc tc

12

<210> 2

<211> 19

<212> DNA

<213> synthetic

<400> 2

atcgactctc gagcggttct

19

<210> 3

<211> 24

<212> DNA

<213> synthetic

<400> 3

tcgtcgaaaa gtcgttttgc tgtt

24

<210> 4

<211> 14

<212> DNA

<213> synthetic

<400> 4

tctcgagcggt tctc

14

<210> 5

<211> 19

<212> DNA

<213> synthetic

<400> 5

tcgactctcg agcgttctc

19

<210> 6
<211> 20
<212> DNA
<213> synthetic

<400> 6

atcgactagc gttcggttctc

20

<210> 7
<211> 16
<212> DNA
<213> synthetic

<400> 7

actctcgagc gttctc

16

<210> 8
<211> 15
<212> DNA
<213> synthetic

<400> 8

ctctcgagcg ttctc

15

<210> 9
<211> 12
<212> DNA
<213> synthetic

<400> 9

gtcgacgttg ac

12

<210> 10
<211> 12
<212> DNA
<213> synthetic

<400> 10

gtcggcgttg ac

12

<210> 11
<211> 18
<212> DNA
<213> synthetic

<400> 11

cgactctcga gcgttctc

18

<210> 12
<211> 12

<212> DNA
<213> synthetic

<400> 12

gtcgacgctg ac

12

<210> 13
<211> 12
<212> DNA
<213> synthetic

<400> 13

gtcagcgttg ac

12

<210> 14
<211> 17
<212> DNA
<213> synthetic

<400> 14

gactctcgag cgttctc

17

<210> 15
<211> 12
<212> DNA
<213> synthetic

<400> 15

gtcgtcgatg ac

12

<210> 16
<211> 20
<212> DNA
<213> synthetic

<400> 16

atgcactctc gagcggttctc

20

<210> 17
<211> 13
<212> DNA
<213> synthetic

<400> 17

ctcgagcggtt ctc

13

<210> 18
<211> 12
<212> DNA
<213> synthetic

<400> 18

tgca~~gcgttc~~ tc

12

<210> 19
<211> 12
<212> DNA
<213> synthetic

<400> 19

tttggcgttt tt

12

<210> 20
<211> 20
<212> DNA
<213> synthetic

<400> 20

atcgactctc gagcgttctc

20

<210> 21
<211> 20
<212> DNA
<213> synthetic

<400> 21

agcg~~tttctc~~ gatcgac~~ctc~~

20

<210> 22
<211> 19
<212> DNA
<213> synthetic

<400> 22

gg~~tg~~caccga tgcaggggg

19

<210> 23
<211> 14
<212> DNA
<213> synthetic

<400> 23

gtcgtcgac~~g~~ acgg

14

<210> 24
<211> 12
<212> DNA
<213> synthetic

<400> 24

gggggcgttg gg

12

<210> 25
<211> 20
<212> DNA

<213> synthetic

<400> 25

atgcactctg cagcggttctc

20

<210> 26

<211> 20

<212> DNA

<213> synthetic

<400> 26

atcgactctc gaggcggttctc

20

<210> 27

<211> 17

<212> DNA

<213> synthetic

<400> 27

ggtgcatcga tgcagggg

17

<210> 28

<211> 25

<212> DNA

<213> synthetic

<400> 28

gggtcgtcgt tttgtcggtt cgttg

25

<210> 29

<211> 12

<212> DNA

<213> synthetic

<400> 29

aaaggcggtta aa

12

<210> 30

<211> 12

<212> DNA

<213> synthetic

<400> 30

cccgccgttc cc

12

<210> 31

<211> 12

<212> DNA

<213> synthetic

<400> 31

gtcatcgatg ca

12

<210> 32
<211> 20
<212> DNA
<213> synthetic

<400> 32

ggtgcatcga tgcagggggg

20

<210> 33
<211> 20
<212> DNA
<213> synthetic

<400> 33

ggggtcatcg atgaaaaaaaa

20

<210> 34
<211> 20
<212> DNA
<213> synthetic

<400> 34

ggtgcatcga tgcagggggg

20

<210> 35
<211> 20
<212> DNA
<213> synthetic

<400> 35

aaggtaaacg ttgaaaaaaaa

20

<210> 36
<211> 20
<212> DNA
<213> synthetic

<400> 36

aaggtaatcg atgggggggg

20

<210> 37
<211> 20
<212> DNA
<213> synthetic

<400> 37

ggtgcatcga tgcagggggg

20

<210> 38
<211> 20
<212> DNA
<213> synthetic

<400> 38

ggtgcatcga tgcagggggg

20

<210> 39

<211> 20

<212> DNA

<213> synthetic

<400> 39

ggtgcgtcga cgcagggggg

20

<210> 40

<211> 20

<212> DNA

<213> synthetic

<400> 40

ggtgcgtcga tgcagggggg

20

<210> 41

<211> 20

<212> DNA

<213> synthetic

<400> 41

ggtgcgtcga cgcagggggg

20

<210> 42

<211> 20

<212> DNA

<213> synthetic

<400> 42

ggtgacccgg tgcagggggg

20

<210> 43

<211> 20

<212> DNA

<213> synthetic

<400> 43

ggtgcatcga tgcagggggg

20

<210> 44

<211> 12

<212> DNA

<213> synthetic

<400> 44

gtcaacgtcg ac

12

<210> 45
<211> 12
<212> DNA
<213> synthetic

<400> 45

gtcggcgctg ac

12

<210> 46
<211> 19
<212> DNA
<213> synthetic

<400> 46

gggtcaacg ttgaggggg

19

<210> 47
<211> 12
<212> DNA
<213> synthetic

<400> 47

gtcggcgctg ac

12

<210> 48
<211> 20
<212> DNA
<213> synthetic

<400> 48

atgcactctc gaggcttctc

20

<210> 49
<211> 17
<212> DNA
<213> synthetic

<400> 49

aatgcacatcga tgcaaaa

17

<210> 50
<211> 12
<212> DNA
<213> synthetic

<400> 50

gtcagcgctg ac

12

<210> 51
<211> 12
<212> DNA
<213> synthetic

<400> 51
gtcaacgttg ac 12
<210> 52
<211> 12
<212> DNA
<213> synthetic
<400> 52
tgcatcgatg ca 12
<210> 53
<211> 19
<212> DNA
<213> synthetic
<400> 53
ggtgcatcga tgcaggggg 19
<210> 54
<211> 12
<212> DNA
<213> synthetic
<400> 54
gtcgacgtcg ac 12
<210> 55
<211> 12
<212> DNA
<213> synthetic
<400> 55
gtcgacgccc ac 12
<210> 56
<211> 12
<212> DNA
<213> synthetic
<400> 56
cccaacgttc cc 12
<210> 57
<211> 12
<212> DNA
<213> synthetic
<400> 57
gtcaacgctg ac 12
<210> 58

10

<211> 10
<212> DNA
<213> synthetic

<400> 58

gagcgttctc

10

<210> 59
<211> 12
<212> DNA
<213> synthetic

<400> 59

gggaacgttg gg

12

<210> 60
<211> 12
<212> DNA
<213> synthetic

<400> 60

gtcagcgctg ac

12

<210> 61
<211> 16
<212> DNA
<213> synthetic

<400> 61

gggggaacgt tcgggg

16

<210> 62
<211> 12
<212> DNA
<213> synthetic

<400> 62

gtcggcgccg ac

12

<210> 63
<211> 16
<212> DNA
<213> synthetic

<400> 63

ggggtaacgt tagggg

16

<210> 64
<211> 12
<212> DNA
<213> synthetic

<400> 64

gtcaacgccc ac

12

<210> 65
<211> 12
<212> DNA
<213> synthetic

<400> 65

tgcctcgagg ca

12

<210> 66
<211> 12
<212> DNA
<213> synthetic

<400> 66

tttaacgttt tt

12

<210> 67
<211> 12
<212> DNA
<213> synthetic

<400> 67

aaaaacgtta aa

12

<210> 68
<211> 16
<212> DNA
<213> synthetic

<400> 68

gggggaagct tcgggg

16

<210> 69
<211> 12
<212> DNA
<213> synthetic

<400> 69

gtcagcgccc ac

12

<210> 70
<211> 11
<212> DNA
<213> synthetic

<400> 70

cgagcgttct c

11

<210> 71
<211> 16

<212> DNA
<213> synthetic

<400> 71

ggtgcatcga tgcagg

16

<210> 72
<211> 20
<212> DNA
<213> synthetic

<400> 72

ggtgcatcga tgcaggggg

20

<210> 73
<211> 19
<212> DNA
<213> synthetic

<400> 73

ggtgcatcga tgcaggggg

19

<210> 74
<211> 13
<212> DNA
<213> synthetic

<400> 74

ggcgtcgacg ggg

13

<210> 75
<211> 19
<212> DNA
<213> synthetic

<400> 75

ggtgcgctgt tgcaggggg

19

<210> 76
<211> 19
<212> DNA
<213> synthetic

<400> 76

ggtgcgccga tgcaggggg

19

<210> 77
<211> 16
<212> DNA
<213> synthetic

<400> 77

gggggatcga tcgggg

16

<210> 78
<211> 13
<212> DNA
<213> synthetic

<400> 78

gggtcgaca ggg

13

<210> 79
<211> 19
<212> DNA
<213> synthetic

<400> 79

ggtgcgtcgg tgcaggggg

19

<210> 80
<211> 16
<212> DNA
<213> synthetic

<400> 80

gggggatgca tcgggg

16

<210> 81
<211> 20
<212> DNA
<213> synthetic

<400> 81

ggtgcgtcga tgcaggggggg

20

<210> 82
<211> 20
<212> DNA
<213> synthetic

<400> 82

ggtgcgtcga tgcaggggggg

20

<210> 83
<211> 19
<212> DNA
<213> synthetic

<400> 83

ggtgcgtcga tgcaggggggg

19

<210> 84
<211> 19
<212> DNA

<213> synthetic

<400> 84

ggtgccctcga ggcaggggg

19

<210> 85

<211> 16

<212> DNA

<213> synthetic

<400> 85

gggggctcga gagggg

16

<210> 86

<211> 16

<212> DNA

<213> synthetic

<400> 86

ggggtatcga tagggg

16

<210> 87

<211> 19

<212> DNA

<213> synthetic

<400> 87

ggtgcacatcga tgcgagaga

19

<210> 88

<211> 19

<212> DNA

<213> synthetic

<400> 88

ggtgcacatcga cgcaggggg

19

<210> 89

<211> 20

<212> DNA

<213> synthetic

<400> 89

ggggtcaacg ttgagggggg

20

<210> 90

<211> 20

<212> DNA

<213> synthetic

<400> 90

ggtgcacatcga tgcagggggg

20

<210> 91
<211> 20
<212> DNA
<213> synthetic

<400> 91

gggggtcaagc ttgagggggg

20

<210> 92
<211> 16
<212> DNA
<213> synthetic

<400> 92

ggggtaagct tagggg

16

<210> 93
<211> 17
<212> DNA
<213> synthetic

<400> 93

ggtgcatgca tgcagggg

17

<210> 94
<211> 20
<212> DNA
<213> synthetic

<400> 94

ggtgcataaa tgcagggggg

20

<210> 95
<211> 17
<212> DNA
<213> synthetic

<400> 95

aatgcatgca tgcaaaa

17

<210> 96
<211> 20
<212> DNA
<213> synthetic

<400> 96

ggtgcatgca tgcagggggg

20

<210> 97
<211> 20
<212> DNA
<213> synthetic

<400> 97

atcgactctg caggcttctc

20

<210> 98

<211> 12

<212> DNA

<213> synthetic

<400> 98

tcgaggcttc tc

12

<210> 99

<211> 20

<212> DNA

<213> synthetic

<400> 99

atgcactctg caggcttctc

20

<210> 100

<211> 12

<212> DNA

<213> synthetic

<400> 100

tcgaggcttc tc

12

<210> 101

<211> 12

<212> DNA

<213> synthetic

<400> 101

tcgttgttgc tc

12

<210> 102

<211> 12

<212> DNA

<213> synthetic

<400> 102

acgagggttc tc

12

<210> 103

<211> 13

<212> DNA

<213> synthetic

<400> 103

ttccttcgag ctc

13

<210> 104
<211> 12
<212> DNA
<213> synthetic

<400> 104

tcgatgcttc tc

12

<210> 105
<211> 12
<212> DNA
<213> synthetic

<400> 105

gcgaggcttc tc

12

<210> 106
<211> 12
<212> DNA
<213> synthetic

<400> 106

ccgaggcttc tc

12

<210> 107
<211> 12
<212> DNA
<213> synthetic

<400> 107

tgcaggcttc tc

12

<210> 108
<211> 12
<212> DNA
<213> synthetic

<400> 108

tcgttcgttc tc

12

<210> 109
<211> 12
<212> DNA
<213> synthetic

<400> 109

tcgcccgttc tc

12

<210> 110
<211> 12
<212> DNA
<213> synthetic

<400> 110

tcgaatgttc tc

12

<210> 111

<211> 12

<212> DNA

<213> synthetic

<400> 111

tcgagtgttc tc

12

<210> 112

<211> 12

<212> DNA

<213> synthetic

<400> 112

tcgtatgttc tc

12

<210> 113

<211> 12

<212> DNA

<213> synthetic

<400> 113

tcggatgttc tc

12

<210> 114

<211> 12

<212> DNA

<213> synthetic

<400> 114

tcgcatgttc tc

12

<210> 115

<211> 12

<212> DNA

<213> synthetic

<400> 115

tcgactgttc tc

12

<210> 116

<211> 12

<212> DNA

<213> synthetic

<400> 116

tcgcctgttc tc

12

<210> 117

<211> 12
<212> DNA
<213> synthetic

<400> 117

tcggctgttc tc

12

<210> 118
<211> 12
<212> DNA
<213> synthetic

<400> 118

tcgtctgttc tc

12

<210> 119
<211> 12
<212> DNA
<213> synthetic

<400> 119

tcgtgtgttc tc

12

<210> 120
<211> 12
<212> DNA
<213> synthetic

<400> 120

tcgcttgttc tc

12

<210> 121
<211> 12
<212> DNA
<213> synthetic

<400> 121

ttgttcgaac tc

12

<210> 122
<211> 12
<212> DNA
<213> synthetic

<400> 122

ttgttcgctc tc

12

<210> 123
<211> 12
<212> DNA
<213> synthetic

<400> 123

ttgttcgccc tc	12
<210> 124	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 124	
ttgttcgggc tc	12
<210> 125	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 125	
ttgttcgttc tc	12
<210> 126	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 126	
ttgttcgtac tc	12
<210> 127	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 127	
tcgagttcgc tc	12
<210> 128	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 128	
tcgagttcgt tc	12
<210> 129	
<211> 12	
<212> DNA	
<213> synthetic	
<400> 129	
tcgagttcga gc	12
<210> 130	
<211> 12	

<212> DNA
<213> synthetic

<400> 130

ctcgtttgtt ct

12

<210> 131
<211> 12
<212> DNA
<213> synthetic

<400> 131

ttcggtttgtt ct

12

<210> 132
<211> 12
<212> DNA
<213> synthetic

<400> 132

cccggtttgtt ct

12

<210> 133
<211> 12
<212> DNA
<213> synthetic

<400> 133

tcgggttggtc tc

12

<210> 134
<211> 11
<212> DNA
<213> synthetic

<400> 134

tgcgcaaggg g

11

<210> 135
<211> 12
<212> DNA
<213> synthetic

<400> 135

tgcgccttgc tc

12

<210> 136
<211> 20
<212> DNA
<213> synthetic

<400> 136

ggtatatcga tatagggggg

20

<210> 137
<211> 20
<212> DNA
<213> synthetic

<400> 137

ggtggatcga tccagggggg

20

<210> 138
<211> 20
<212> DNA
<213> synthetic

<400> 138

ggtccatcga tccagggggg

20

<210> 139
<211> 20
<212> DNA
<213> synthetic

<400> 139

ggtggatcga tggagggggg

20

<210> 140
<211> 11
<212> DNA
<213> synthetic

<400> 140

agcgctaggg g

11

<210> 141
<211> 20
<212> DNA
<213> synthetic

<400> 141

ggtgcatgta tgcagggggg

20

<210> 142
<211> 20
<212> DNA
<213> synthetic

<400> 20

ggtgcacgcg tgcagggggg

20

<210> 143
<211> 13
<212> DNA

<213> synthetic

<400> 143

cgttctcggg ggg

13